Analytical Studies on Solanesol-Like Substances in Tobacco

Solanesol, an acyclic trisesquiterpenoid alcohol of tobacco (1, 2), has been used as a source of isoprene units in the synthesis of certain metabolically active quinones (3, 4). The lengthy total synthesis of solanesol recently published (5) may be economically impractical, and commercial use of cheap tobacco, including waste, as a source of solanesol remains a possibility.

At present, no analytical data are available in the literature on the amounts of the terpene found in various tobacco types and grades. This information would be of value not only in studies on possible chemurgic use but also in determining chemical composition-tobacco quality relationships, a field of considerable academic and commercial interest.

The development of an analytical method for solanesol in cured, aged, or fermented tobaccos presents complications not frequently encountered in determinations of other plant constituents. There is indirect evidence that small amounts of olefinic analogues or isomers of solanesol (6) and oxidation products of the terpene (7–9) may occur in such leaves. Although the adopted procedure was designed to reduce the contribution of such substances, it was felt desirable to regard the method as a determination of "solanesol-like substances" (SLS) rather than "solanesol."

Experimental

Preliminary.—Initial attempts at colorimetric determination using the Liebermann-Burchard, Carr-Price, or Craig, *et al.* (10) reactions failed. Although *p*-dimethylaminobenzaldehyde reacted with pure solanesol to give a useful concentration-absorption relationship, interfering substances in tobacco extracts prevented valid determination. Attempts to separate the interfering substances by solvent extraction or paper chromatography failed. Likewise, gravimetric and vapor phase chromatographic determinations were unsuccessful, although the latter was not extensively studied.

A satisfactory procedure was ultimately de-veloped; this procedure involves chromatographic separation of *n*-hexane extracts on alumina and infrared absorption measurements of SLS. The spectra of the eluates containing SLS are almost identical with pure solanesol except for the presence of weak to moderate carbonyl bands. Deviations from this pattern are discussed below.

Extraction and separation procedure.—A 12.5 g sample of tobacco ground to pass a 50-mesh screen is extracted in a Soxhlet apparatus with 250 ml of Skellysolve B[1] for 24 hours. The solvent is evaporated on a steam bath under nitrogen and the residue is then disolved in 10 ml of 60% benzene in petroleum ether. This solution is immediately added to a 1½ × 24″ chromatographic column of Merck alumina (100 g). The column is developed by the addition of 5 L of 60% benzene in petroleum ether followed by 5 L of benzene; each solvent is collected separately, and the 60% benzene eluate is discarded. The benzene eluate, which contains the SLS, is evaporated to a residue, weighed, and analyzed as follows:

Infrared spectrophotometric analysis.—The procedure is based on the extinction of the sample at 10.1 (allylic alcohol) and 12.0μ ($R_1R_2C = CHR_3$) compared to a standard, using the baseline technique of Heigl, *et al.* (11). The anchor points for baseline correction of the 10.1μ band are 9.5 and 10.8μ; those for the 12.0μ peak are 11.1 and 13.0μ (Fig. 1).

Standard calibration curves of percentage concentration *vs.* corrected absorbance at 10.1 and 12.0μ are prepared for authentic solanesol at 0.5, 1.0, and 2.0% in CS_2 with matched 1.0 mm NaCl cells and a Perkin-Elmer Model 137 (Infracord) spectrophotometer. A complete infrared spectrum ($2.5–15.0\mu$) of a 2% solution in CS_2 of the residue from the benzene eluate is determined. The corrected absorbances at 10.1 and 12.0μ are calculated, and values for percentage of SLS in the solution at the two wavelengths are determined independently from the corresponding standard curves. The average percentage SLS in the residue (P_E) is

$$P_E = 25 \ (C_{10.1} + C_{12.0})$$

where $C_{10.1}$ and $C_{12.0}$ are the concentrations

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[1] Mention of a specific commercial product does not constitute endorsement by the U.S. Department of Agriculture.

(percentage) of SLS in the CS_2 solution determined from the 10.1 and 12.0μ peaks, respectively. The percentage solanesol in the leaf is then calculated from the weights of eluate and the tobacco extracted.

The use of calibration curves is desirable, since a slight deviation from Beer's Law is observed. The averaging of the absorption bands for hydroxyl and unsaturated groups compensates, in some degree, for the possible presence of small amounts of olefinic analogues of solanesol in the extracts. Also, this procedure reduces the contribution of any extraneous alcoholic or unsaturated groups which may be present in amounts sufficient to alter the quantitative results but insufficient to affect the ratio of absorbances (see below).
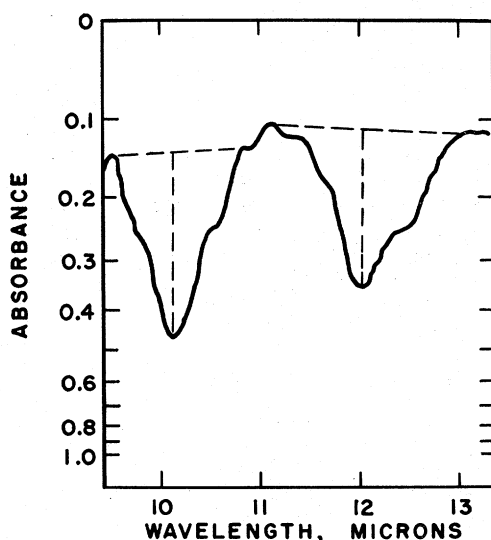

Fig. 1—Infrared absorption curve of benzene eluate showing baseline correction technique.

Recovery experiments showed that 97 and 100%, respectively, of added solanesol were recovered in duplicate runs. Reproducibility of the procedure is approximately ± 5%.

The authentic solanesol (m.p. 35–36°C, Fisher-Johns) was kindly provided by Dr. R. L. Rowland, R. J. Reynolds Tobacco Company, and gave the following analyses: C, 85.50; H, 12.11; 8.9 double bonds per mole. $C_{45}H_{74}O$ requires C, 85.64; H, 11.82; and 9.0 double bonds per mole.

Miscellaneous methodology.—The basis of the method is that the measured absorption is due exclusively to SLS. In spite of the similarities in infrared spectra between authentic solanesol and benzene eluates, it was

recognized that extraneous substances may influence absorption at either 10.1 or 12.0μ. To detect such interference, the ratio of absorbances at the two wavelengths ($A_{12.0}/A_{10.1}$) was calculated in each instance and compared with the range of ratios for authentic solanesol: 0.75 ± 0.05.

The absorbance of the carbonyl band at 5.8μ was also measured to obtain further information on differences among tobacco types and grades.

By crystallization of benzene eluates from acetone at −25°C, a crude approximation of the amount of solanesol in the SLS can be made. A benzene eluate derived from burley tobacco was analyzed and found to contain 87% SLS. Crystallization of the eluate yielded approximately 70% crude solanesol, m.p. 30–31°C, indicating that a large part of the SLS is solanesol. However, the complexity of chemical composition of hexane extracts (12) dictates adherence to the description, "solanesol-like substances."

Table 1. Content of solanesol-like substances in aged or fermented tobaccos

Tobacco	SLS Content (%)		$(A_{12.0}/A_{10.1})^a$
	In Benzene Eluate	In Tobacco	
Bright	85	1.71	0.76
Burley, Sample A	90	1.77	0.79
Burley, Sample B	87	1.71	0.78
Maryland	78	0.85	0.75
Turkish, Sample A	72	0.59	0.77
Turkish, Sample B	47	0.19	0.71
Fire-cured	66	0.72	0.73
Cigar binder (Conn.)	60	0.38	0.71
Cigar filler (Pa.)	75	0.44	0.79
Perique	78	0.81	0.78

^{a} Ratio of absorbances for 10.1μ and 12.0μ bands. Authentic solanesol = 0.75 ± 0.05.

Results and Discussion

Aged or Fermented Tobaccos.—Comparative studies were made of the levels of SLS in representative commercial samples of aged or fermented tobaccos. The samples were the same as those used in a recent study on the higher fatty acids of tobacco (13). The findings are presented in Table 1. No corrections for moisture were made, since all samples contained essentially the same moisture levels, 10%.

Burley and bright tobaccos have levels

of SLS greater than 1%. Maryland, Perique, fire-cured, cigar filler, and cigar binder contain less SLS. The two samples of Turkish tobacco showed significant differences. Sample B had an infrared spectrum which deviated somewhat from authentic solanesol, mainly in the shape of the 10.1μ band. Considering the high percentage (53%) of material in the benzene eluate not responding to the analysis, this deviation is perhaps not unexpected. The Turkish samples were obtained from two different organizations; Sample A was described as "Nigrita, Greece," and no description of Sample B was provided.

All samples gave ratios of absorbances for alcoholic and unsaturated groups within the range of values for authentic solanesol.

Of all tobaccos examined, only Latakia failed to give an analytical value. The infrared spectrum of the benzene eluate was quite different from that of other tobaccos and pure solanesol, although the differences could be attributed in part to other substances obscuring the absorbance of small amounts of SLS in the eluate. The predominate feature was a broad, moderately strong band (absorbance = 0.638) in the carbonyl region, beginning at 5.6μ and merging with the 6.1μ band for unsaturation.

Table 2 shows the absorbance due to carbonyl-containing material accompanying the solanesol-like substances. In general, an inverse relationship exists between the content of such material and the levels of SLS in the benzene eluates, although bright and Turkish B were exceptions.

Variations in SLS of Various Grades, U.S. Types, and Crops.—Commercial samples of unaged, flue-cured (bright) tobacco representing two U.S. Types, two crops (1957, 1958), and three grades (low, medium, and high qualities) were studied. The grades were judged by the organization providing the samples and were identical with those previously used in the study on higher fatty acids. The findings are presented in Table 3.

No relationship between tobacco quality and content of SLS was evident. The levels of SLS were within the general range found for aged burley and bright in two of the three sets of samples. Type 12 showed

Table 2. Absorbance of carbonyl-containing material accompanying solanesol-like substances

Tobacco	Absorbance[a] at 5.8μ	Relative Absorbance (BurleyA = 1.00)	% SLS in Benzene Eluate
Bright	0.280	2.39	85
Burley, Sample A	0.117	1.00	90
Burley, Sample B	0.131	1.12	87
Maryland	0.181	1.55	78
Turkish, Sample A	0.222	1.90	72
Turkish, Sample B	0.237	2.03	47
Fire-cured	0.469	4.01	66
Cigar binder (Conn.)	0.658	5.62	60
Cigar filler (Pa.)	0.222	1.90	75
Perique	0.215	1.84	78

[a] Of a 2% solution in CS_2 with a 1.0 mm NaCl cell.

much higher concentrations; studies on other samples of unaged, cured Type 12 of a different year indicated that such high concentrations are atypical for this type. Considering the profound effect of rainfall, cultural practice, and other factors not considered here, no explanation of this difference can be offered from the available data.

The ratios of absorbances for hydroxyl and unsaturated groups were within the range of authentic solanesol. The absorbances for carbonyl-containing groups were similar for two of the three sets, the odd set having relatively high absorbance but intermediate levels of SLS. This again illustrates the complex differences between grades, types, and crops, and the paucity of detailed information on the chemical substances which are responsible for such differences in tobacco.

Further Studies.—Esterified solanesol has been isolated from flue-cured tobacco (14) and smoke (15) in amounts equivalent to less than 0.1% of the leaf. Glycosidated solanesol has not been reported. An attempt was made to determine total SLS with the above procedure after acid hydrolysis and/ or saponification by the methods previously described for total phytosterols (16). The saponified sample was 35% lower than the unsaponified sample; this result indicates some destruction of material responding to the analysis. The sample which was acid-

Table 3. Content of solanesol-like substances in unaged, flue-cured tobacco of various U.S. Types, years, and qualities

U.S. Type	Year	Quality	SLS Content in Tobacco (%)	$(A_{12.0}/A_{10.1})^a$	Absorbance[b] at 5.8μ
12	1957	Low	2.50	0.79	0.119
		Medium	2.39	0.75	0.108
		High	2.32	0.79	0.105
13	1957	Low	1.65	0.80	0.219
		Medium	1.93	0.75	0.237
		High	1.45	0.78	0.244
13	1958	Low	1.26	0.80	0.100
		Medium	1.33	0.74	0.108
		High	1.21	0.79	0.114

[a] See Table 1, footnote.
[b] See Table 2, footnote.

hydrolyzed and saponified showed even greater destruction, and no valid estimate of total SLS could be made.

Two samples of midribs from unaged, flue-cured tobacco were also studied. The residues from the benzene eluates were relatively low in weight and contained insufficient SLS to yield a valid analytical figure. Based on the weights of residues and assuming less than 10% SLS in the residues, the SLS levels in such midribs were less than 0.04%.

Acknowledgments

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